PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Good HPLC Practices	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down procedure for good HPLC practices.

2.0 SCOPE:

This SOP is applicable to HPLC general practices while performing analysis.

3.0 RESPONSIBILITY - Execution - Executive QC

Checking - Assistant Manager QC

4.0 ACCOUNTABILITY - Manager Quality Control

5.0 PROCEDURE:

5.1 Preparation of Mobile phase and usage of solvents

5.1.1 Use HPLC grade solvents (In case of non-availability of HPLC grade solvents use AR grade solvents) and water for injection or any high purity water (suitable for HPLC analysis) for preparation of mobile phase.

5.1.2 Preparation of buffer solution: If mobile phase contains buffer solution, first calculate the quantity of mobile phase required for complete analysis (including quantity required for dilution) and prepare the buffer solution as per the procedure.

5.1.3 Set the pH. (If required)

5.1.4 Filter the buffer solution if required through 0.45µ / 0.2µ nylon filter.

Do not filter if filtration required in final mobile phase composition .

5.1.5 Measure the aliquot of buffer solution required in the mobile composition and transfer to a clean and dried stopper bottle.

5.1.6 Measure the require quantity of organic solvents separately and add to the stoppered bottle containing buffer solution and mix well.

5.1.7 If mobile phase contains small amounts (5% or less) of solvents, use a volumetric flask /pipette / measuring cylinder of appropriate size for the measurement.

5.1.8 Filter the mobile phase through nylon 0.45 µ or 0.2µ filter. Degas by sonication or vacuum for at least 5 minutes.

5.1.9 Make entry of mobile phase preparation in the “Mobile phase preparation and system suitability” format.(Attachment-1)

5.1.10 If it requires to store the mobile phase for more than a day, first degas mobile phase and immediately stopper the bottle, wrap Para film and keep bottle at room temperature.

5.1.11 Use stored mobile phase / buffer solution within 3 days.

5.1.12 Before the usage of stored 'mobile phase /buffer solution', check the pH first (if applicable) and adjust if required, make the entry of the same in "Remark" column of "Mobile phase preparation and system suitability format". Then filter, degas and use.

5.2 System set and Usage of column:

5.2.1 Low the instrument operating procedure and make necessary entry in the instrument usage log.

5.2.2 Attach the column and make necessary entry in the "Column usage log".

5.2.3 Make entry of "Chromatographic condition" Columns details and HPLC set-up in "Mobile phase preparation and System suitability format"(Attachment-1)

5.2.4 Wash the column with storing solvent and with mediate solvent before the mobile phase.

5.2.5 Saturate the column with mobile phase for about 30 mins. Or more until baseline gets stabilized or as specified in the individual Monograph /test procedure.

5.2.6 Inject System suitability solution / Standard solution and set the appropriate integration parameters.

5.2.7 Check the peak shape, retention time, resolution, asymmetry and theoretical plates etc. from the first injection of System suitability solution or Standard preparation and if require make necessary modification in system.

5.2.8 Change auto sampler rinsing solvent or in mobile phase as per step 5. 3 & 5.4 for the system suitability.

5.2.9 Start Sample analysis only if, the system suitability parameters are within specified limits.

5.2.10 Fill the details of System suitability parameters in the "Mobile Phase" preparation and System suitability format" (Attachment-I).

5.2.10 After the completion of analysis, wash the column with mobile phase, mediate solvent and storing solvent, do auto injector rinsing and purging and make necessary entry in the "Column Usage log" and in "Instrument Usage log".

5.3 Unless otherwise specified in the test procedure /template /pharmacopoeia follow the given below parameters for System suitability.

5.3.1 Relative standard deviation for 5 replicate injections of standard preparation should not be more than 2.0 %.

5.3.2 Relative standard deviation for triplicate injection of standard preparation of standard preparation should not be more than 1.0 %.

5.4 Allowable modification in Chromatographic system Following after the general criteria which provides the extent of allowable variation to get the system suitability. The adjustments are allowed only to improve the quality of the chromatography where no specific requirement is provided.

5.4.1 pH of the aqueous component (Buffer) of the mobile phase can be adjusted by ± 0.2pH. (Ex.: If specified pH is 7.0 then allowable limit for adjustment is 6.80 -7.20).

5.4.2 Concentration of salts in the buffer component of a mobile phase cab be adjusted by ± 10%pH. (If specified concentration is 1.0% then allowable limit for adjustment is 0.9%-1.0%).

5.4.3 Stationary phase: column length: ± 70 %. (Ex.: If specified length is 25 cm then allowable limit for adjustment is 7.5cm - 42.5cm.)

5.4.4 Column internal diameter: ± 25%. (Ex.: If specified internal diameter is 4.6 mm then allowable limit for adjustment is 3.45-5.75mm.).

5.4.5 Particle size: Maximum reduction of 50%, No Increase permitted. (Ex.: If specified particle size is 5micron then allowable maximum reduction is 2.5 micron.)**.**

5.4.6 Flow rate: ± 50%. (If specified flow rate is 1ml /min. then allowable limit for adjustment is 0.5 -1.5ml/min.).

5.4.7 Column temperature: ± 10%, to a maximum of 60 ° C. (Ex.: If specified column temperature is 40° C then allowable limit for adjustment is 36° -44° C.)

5.4.8 Detector wavelength: No adjustment permitted.

5.4.9 Injection volume can be reduced but care should be taken that it should not affect detection of peaks and repeatability of area. Injection volume may be increased to as much as twice the volume specified but care should be taken that it affect adversely to baseline , peak shapes, resolution , and retention time.

5.4.10 Mobile phase composition can be changed as follows

Minor component ± 30%, or ± 2% absolute (In relation to total volume of mobile phase). Which ever is larger. Change in any component not more than 10% absolute nor the final concentration of any component be reduced to zero. Examples of adjustments are given below.

5.4.10.1 If the specified ratio is 50:50 then, thirty percent of 50 is 15% absolute, but this exceeds the maximum permitted change of ±10% absolute in either component, therefore the mobile phase ratio may be adjusted only within the range of 40: 60 to

5.4.10.2 If the specified ratio is 95:50 then, thirty percent of 5 is 1.5% absolute, however because adjustments up to ± 2% absolute are allowed, the ratio may be adjusted within the range of 93:7 to 97:3.

5.4.10.3 If the specified ratio is 2:98 then, thirty percent of 52is 0.6% absolute. In this case an absolute adjustment of –2% is not allowed because it would reduce the amount of the first component to zero. Therefore the maximum allowed adjustment is within the range of 1:4:98.6 to 2.6:97.4.

5.4.10.4 If the specified ratio is 60:35:5 then, for the second component, 30% of 35 is10.5% absolute, which exceeds the maximum permitted change of ±10% absolute in any component. Therefore the second component may be adjusted only with in the range of 25% to 45% absolute.

5.4.10.5 For the third component, 30% of 5 are 1.5% absolute. Since ±2% absolute is permitted and provides more flexibility, the third component may be adjusted within the range of 3% to 7% absolute. In all cases, a sufficient quality of the first component is used to a total of 100%.

5.5 HPLC Injection Scheduling

Following are the injection scheduling guidelines, which can be, alter as per the monograph or any specific requirement.

5.5.1 Stage: In-process

Sr. No.	Sample Id	No. of Injection	Remarks	System suitability Criteria
1	Blank	1	To be observe for any peak at RT of Principal peak	No peak at RT of Principal peak.
2	Resolution or System Suitability	1	If finished product /raw material/stability samples are to be clubbed	For the confirmation of peak resolution or System Suitability Criteria
3	Standard	1	If finished product /raw material/stability samples are to be clubbed inject 5 Standard preparation	RSD: NMT 1.0% (For 3Injections)&NMT2.0% (for 5 Injections)

Sr. No.	Sample Id	No. of Injection	Remarks	System Suitability Criteria
4	Sample -1	2	For“Assay”duplicate injections of each sample preparation. For “Dissolution”& “Content Uniformity “ single injection of each sample preparation.	
5	Sample -1	2		
6	Sample -1	2		
7	Sample -1	2		
8	Sample -1	2		
9	Sample -1	2		
10	Standard	2	Bracketing standard. For calculation of sample Nos. 01 to 06 consider mean area of standard Injection Nos. 4,5,6&. OR Carry out after 300 min. of sample run time.	RSD: NMT 2.0% for the Standard 4,5,6and 7 in case of raw material / finished product or stability samples clubbed.

5.5.2 Stage: Trial Study

Sr. No.	Sample Id	No. of Injection	Remarks	System Suitability Criteria
1	Blank	1	To be observed for any peak at RT of Principal peak	No peak at RT of Principal peak
2	Resolution or System Suitability Solution	1	For the confirmation of peak resolution or System Suitability Criteria	As per requirement.
3	Standard	3 or 5	System Suitability check	RSD: NMT 1.0% (FOR 3 Injections) & NMT 2.0% (For 5 injections)
4	Sample -1	2	For"Assay"duplicate injections of each sample preparation. For"Dissolution"& "Content Uniformity " single injection of each sample preparation	
5	Sample - 2	2		
6	Sample - 3	2		
7	Sample - 4	2		
8	Sample - 5	2		
9	Sample - 6	2		

5.5.3 Stage: Raw material / Finished product / stability

Sr. No.	Sample Id	No. of Injection	Remarks	System Suitability Criteria
1	Blank	1	To be observed for any peak at RT of Principal peak	No peak at RT of Principal peak
2	Resolution or System Suitability solution	1	For the confirmation of peak resolution or system Suitability Criteria	As per requirement
3	Standard	5	System Suitability check	RSD: NMT2.0 % & parameters to comply as per ATP / Pharmacopoeia.

Sr. No.	Sample Id	No. of Injection	Remarks	System Suitability Criteria
4	Sample -1	2	For "Assay","Dissolution"& "Assay"+ Content Uniformity" maximum 10 injections, 12 injections & 12 injections of sample respectively should be bracketed by duplicate injection of standard preparation .For "Dissolution" and "Content uniformity" single injection of sample preparation to be done.	Should meets the Requirements as per the test procedure / pharmacopoeia
5	Sample - 2	2		
6	Sample - 3	2		
7	Sample - 4	2		
8	Sample - 5	2		
9	Sample -6	2		
10	Standard	2	Bracketing standard. For calculation of sample nos. 01 to 06 consider mean area of standard injection nos. 4,5,6,&7. OR Carry out after 300min. of sample run time.	RSD : NMT 2.0% for the standard 4,5,6, and 7.
11	Sample -7	2	For Raw material, Stability samples and finish product each 5-sample preparation (i.e.10 sample injections) or 300min. of sample total run time (inject as per which one minimum requirement) should be bracketed by duplicate standard injection.	
12	Sample -8	2		

Sr. No.	Sample Id	No. of Injection	Remarks	System suitability Criteria
13	Sample - 9	2		
14	Sample -10	2		
15	Sample - 11	2		
16	Sample - 12	2		
17	Standard	2	Bracketing standard. For calculation of sample nos. 07 to 12 consider Mean area of standard injection nos. 6,7,8 & 9 or Carry out after 300 min. of sample run time.	RSD: NMT 2.0% FOR THE Standard 6,7,8and 9
In case of more injections are required, follow the above procedure.				

5.5.4 Stage: For Stability / Finished product degradation test

Sr. No.	Sample Id	No. of Injection	Remarks	System Suitability Criteria
1	Blank	1	To be observed for any peak at RT of Principal peak	No peak at RT of Principal peak
2	Placebo	1	To identify the placebo peak /s.	----------
3	Sample - 1	2	For the degradation calculation	
4	Sample - 2	2		
5	Sample - 3	2		
6	Sample - 4	2		
7	Sample - 5	2		

5.5.5 Stage: FP /RM for Related substances / Chromatographic purity

Sr. No.	Sample Id	No. of Injection	Remarks	System suitability Criteria
1	Blank	1	To be observed for any peak at 1 RT of Principal peak	No peak at RT of Principal peak
2	Resolution solution	1	System Suitability Check	As per the ATP/ Pharmacopoeia
3	Placebo	1	To confirm the peaks of Placebo.	--------
4	Lowest concentration solution	1	Set the sensitivity / peak Response using lowest concentration as defined in procedure.	Peak obtained from lowest concentrated solution should detect and quantitate.
5	Diluted test solution	3	System Suitability check .If more than one sample lots are to be analyzed then inject diluted test solution of any one lot and identify it properly.	RSD: NMT3.0%(For 3 Injection) & NMT 5.0% (For 5 injection) & other parameter s to comply as per test procedure / pharmacopoeia.
6	Test solution - 1	2	Calculate the impurity % by comparing the area with that obtained from diluted test solution and / or Specific impurity solution.	
7	Test solution - 2	2		
8	Test solution - 3	2		

5.5.6 Above mention injection scheduling is providing guideline for injection pattern , but changes in schedule can be allowed , like additional injection of placebo, blank and system suitability solution (resolution)at end of sequence or in- between sequence.
In case of vial missing /area variation found /additional peak observed /, re injection from the same vial or from the same solution can be done.

5.5.7 No standard bracketing for in- process sample is required but if finished product or other samples are clubbed with in-process sample then follow standard bracketing, requirement as per 5.5.3.

5.5.8 For the analysis of validation samples like process validation, cleaning validation (Swab sample and mock sample) follow the injection pattern of “finished Product” except single sample injection for cleaning validation sample preparation.

5.5.9 For In- process sample if RSD of triplicate Standard injection is not within the limit then inject two more standard injections and calculate the RSD of five replicate injection and RSD limit shall be NMT 2.0%.

5.6 Checking of chromatograms

5.6.1 In the blank / placebo chromatogram identify the diluents /placebo peak if present and exclude the dilutes / placebo peak area for impurity calculation in related substances and chromatographic purity test.

5.6.2 Check that the integration parameters like width, threshold values are selected appropriately for proper peak marking and detection.

5.6.3 In the System Suitability chromatogram identify the peaks, its RRT , tailing factor , peak shape and report the values as applicable.

5.7 Calculations and Documentation

5.7.1 The custom report shall cover the following information, which is for information and can be modify as per the specific need.

5.7.1.1 Name of product /Raw Material

5.7.1.2 Test performed

5.7.1.3 B.No. / A.R.No.

5.7.1.4 Method path

5.7.1.5 Sequence path

5.7.1.6 Data path

5.7.1.7 Injection volume

5.7.1.8 Vial No.

5.7.1.9 Date of acquisition

5.7.1.10 Name of instrument used

5.7.1.11 Analyst

5.7.1.12 Page No.

5.7.2 The peak table in custom report shall cover the following data, however select other data as per requirement.

5.7.2.1 Peak No.

5.7.2.2 Name

5.7.2.3 Retention time

5.7.2.4 Area

5.7.2.5 Area %

5.7.2.6 Tailing factor or Asymmetry.

5.7.2.7 Theoretical plates

5.7.2.8 Integration codes

5.7.2.9 Resolution (If required)

5.7.3 Ensure System suitability parameters from the first standard injection and on completion of specified number of standard injections calculate the system suitability parameters on last standard chromatogram. Record the values in the "Mobile phase preparation and system suitability " format.

5.7.4 In case of analysis discontinuation, mention the reason for discontinuation in usage Log.

5.7.5 Take print out of integration on system suitability chromatogram (last Standard chromatogram).

5.7.6 Documentation related to "HPLC' chromatogram check list" format

5.7.6.1 Put the " " mark indicates the completion of that stage or put'NA' where it is not applicable.

5.7.6.2 If any parameter in HPLC chromatogram check list is not comply or not carried out take authorization of Manager Quality Control.

5.7.6.3 Analysis shall be done as per the guideline provided in the "HPLC" chromatogram Checklist" format.

5.7.6.4 Document any deviation in QC-Deviation formats and take authorization of Manager Quality Control.

5.7.6.5 Put "HPLC DATA" stamp on the first chromatogram of actual Analysis (i.e. Blank) and attach duly filled "Mobile phase preparation and system suitability" format (If required), "HPLC" chromatogram check list" format and "sequence print out " along with the chromatograms.

5.7.6.6 Put all chromatograms together and attach with the relevant batch No. /A.R. No. document.

5.7.6.7 In case where more than one batch /lot of sample are clubbed.

5.7.6.8 For analysis, attach all chromatograms with any one batch /A.R. No. document and give cross reference in all other batch / A.R.No. documents.

5.8 General guidelines

5.8.1 Column change in between the analysis

5.8.1.1 Put remark on the last chromatogram of the previously used column regarding change over reason and change over column no.

5.8.1.2 Make entry in the column usage logbook and keep the previously used column for washing.

5.8.1.3 Put remark for discontinuation of the column in the previously used column usage log.

5.8.1.4 Preserve all chromatograms in serial and attach with the analytical report. DO NOT DESTROY ANY CHROMATOGRAM OBTAIN WITH THE PREEVIOUS COLUMN.

5.8.2 Mobile phase change over &Re –preparation of Mobile phase

5.8.2.1 Attach both the mobile phase preparation formats with the chromatograms.

5.8.2.2 Put remark for the discontinuation of the mobile phase.

5.8.2.3 Preserve all the chromatograms obtained from first mobile phase and put remark "Trial" on the chromatograms.

5.8.3 Run time and replicate injection

5.8.3.1 In the test for Assay, run all Standard and sample chromatograms about5 minutes extra after the principal peak elution is over and peak is properly integrated.

5.8.3.2 In chromatographic Purity /Degradation /related substances /stability samples analysis, run the chromatogram 2.5 times the RT of principal peak or as specified in individual ATP /Pharmacopoeia. In case of specific impurity analysis , run the chromatogram as specified in monograph.

5.8.3.3 In the test for related substances /Chromatographic purity / degradation product where the impurity % is to be calculated by area normalization method only from the sample preparation.

5.8.3.3.1 Inject sample preparation in triplicate and calculate the system suitability parameters specified in the procedure. Inject first sample preparation in triplicate for system suitability check, then after inject duplicate injection for every additional sample analysis.

5.8.3.3.2 Calculate system suitability parameters if specified in the procedure.

5.8.3.3.3 If RSD of triplicate injection is not less than 2.0 % then inject another 2 injections of sample preparation and calculate the RSD of principal peak, it should be below 3.0%.

5.8.3.3.4 Calculate the % Impurity from all the sample injections.

5.8.3.4 If degradation /related substance is to be calculated from Assay, take separate print out of sample chromatogram by setting the width and threshold appropriately to detect the all peaks.

5.8.3.5 In the test for Degradation /Chromatographic purity, Identify the peak in the chromatogram by blank, Placebo, "Active Content", known impurity (if any) & unknown impurity in the chromatogram of sample preparation.

5.9 Check the raw data as mentioned in Annexure - II.

6.0 SAFETY & PRECAUTIONS:

Not applicable.

7.0 REVISION HISTORY:

Revision No.	Reason for Revision	Superseded from & Date

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8.0 DISTRIBUTION:

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9.0 REFERENCES:

USP and BP.

10.0 ABBREVIATIONS & ANNEXURES:

SOP : Standard Operating Procedure

QC : Quality Control

USP : United States Pharmacopoeia

BP : British Pharmacopoeia

No. : Number

Annexure - I: Mobile Phase Preparation And System Suitability Format

Annexure - II: HPLC Chromatogram Checklist

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ANNEXURE - I

MOBILE PHASE PREPARATION AND SYSTEM SUITABILITY FORMAT

Product- B.No. / A.R. No. -

Test- Method Ref. - BP/EP /USP/IH

Parameters	Test Condition	Applied Condition	Sign/ Date	Mobile Phase Preparation	Sign/ Date
1.0 - Chromatographic Condition - Column No.				Buffer Preparation	
a. Column make and Type				_____gm/ml of _____ + _____ gm/ml of _____ + ___ gm/ml of ___ taken in ___ ml Volumetric flask / beaker , added _____ ml of ml of water /,dissolved by sonication /hand shaking for _____min. and diluted to _____ml with _____and mix. Initial pH: _____ pH adjusted to _____ using _____ ml of _____ (Limit:)	
b. Column dimensions					
c. Column temperature					
d. Auto sampler temperature					
e. Wave Length					
f. Injection volume					
g. Other (If any)					
2.0 -Integration Parameters					
a. Width					
b. Threshold / Slope sensitivity					
c. Integration off					
d. Valley to Valley					
e. Other (If any)					
3.0 - System suitability					
Reference Chromatogram No. -					
a. RSD					
b. Theoretical plates					
c. Tailing factor					
d. Resolution				Degassed by sonication /vacuum for mins	

Filtered through _____ µ (0.45/0.22µ) filter (If required)		
Composition and Sequence of addition: (Total quantity prepared __ ml)		
Buffer	Solvent _____	
	Acetonitrile	Methanol
Mixed for ___ mins, on stirrer/ sonicator / shaking		
Filtered through 0.45/0.22µ filter.		

Remarks:

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ANNEXURE - II

HPLC CHROMATOGRAM CHECKLIST

Product/Sample - _____ A.R. No. - _____

Test- _____ Batch No. - _____

Reference -

(H.B. No./Protocol No./Template No.) Page No. - _____

1. Integration Parameters should be printed on last standard chromatogram of System Suitability or attach separate sheet. ☐
2. Chromatograms should be in serial of blank, Standard and Sample from the first Injection (including trials). If not, should be authorized. ☐
3. Cancellation of any chromatogram should be justified and authorized. ☐
4. Each chromatogram should be properly identified. ☐
5. Chromatograms should be properly identified. (e.g. trial ,Blank, Standard ,Sample) ☐
6. System Suitability Parameters should be filled in the respective format and should be within limit, before Sample injection. ☐
7. In Chromatographic Purity /Degradation / Related Substances /Stability samples analysis, run the chromatogram 2.5 times the RT of principal peak or as specified in individual ATP /template /protocol /Pharmacopoeia. ☐
8. First Chromatogram of actual analysis (blank) should be stamped with “HPLC data” STAMP. ☐
9. For single test, Method and integration parameters should be same through out the analysis. ☐
10. Any type of Reintegration should be authorized. ☐
11. Samples should be bracketed by Standards as per the system. If not, should be authorized

Remarks:

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